This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claims 1-49(canceled).

50(new). An assay method for the determination of holo-TranscobalaminII (holo-TCII) in a body sample, comprising contacting a cell free sample of a body fluid with an immobilized cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of TCII and haptocorrin (HC) in said sample over the holo-forms thereof, subsequently contacting said sample which has been contacted with the immobilized cobalamin or analogue or fragment thereof, with a specific binding ligand for TCII or holo-TCII, separating a ligand bound fraction from a non-ligand bound fraction and measuring the TCII or cobalamin content of said ligand bound fraction to determine the quantity of holo-TCII in the body sample being assayed, wherein the binding of said apo-TCII to said immobilised cobalamin or analogue or fragment thereof is followed by removal of said apo-TCII or renders said apo-TCII unable to bind to said specific binding ligand for TCII or holo-TCII.

51(new). An assay method as claimed in claim 50 wherein the separation of said ligand bound fraction from said non-ligand bound fraction is so performed that the holo-TCII concentration is increased by at least 3-fold. previous 29

52(new). An assay method as claimed in claim 50 wherein said assay is capable of detecting holo-TCII at a concentration as low as 9 pM. p 30

53(new). An assay method as claimed in claim 50 wherein said specific binding ligand is a ligand selected from the group consisting of a polyclonal antibody, a monoclonal antibody, and an antibody fragment. p 31

54(new). An assay method as claimed in claim 50 wherein said specific binding ligand exhibits a high degree of selectivity and specificity towards TCII and exhibits low affinity towards other transcobalamin proteins, in either apo or holo form, or any other cobalamin-binding protein. p 32

55(new). An assay method as claimed in claim 50 wherein cobalamin in said ligand bound fraction is released from the holo TCII molecules therein by changing the temperature or the pH of the surrounding medium. 33

56(new). An assay method as claimed in claim 55 wherein said released cobalamin is determined by a competition assay performed by contacting an immobilised binding partner for cobalamin with the released cobalamin of the sample in the presence of labelled cobalamin analogue which competes with the released cobalamin for binding to the immobilised binding partner. 34

57(new). An assay method as claimed in claim 50 wherein said method comprises contacting a solid support having immobilised thereon said specific binding ligand for TCII or holo-TCII, with a non-immobilised ligand and also with the sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof,

wherein said immobilised ligand binds to TCII or holo-TCII, to said non-immobilised ligand or to complexes of said TCII or holo-TCII and said non-immobilised ligand, and said non-immobilised ligand binds to at least one of said immobilised ligand, TCII or holo-TCII and complexes of said immobilised ligand apo-TCII or holo-TCII; 35

wherein said assay method is a sandwich assay and at least one of said ligands is specific for holo-TCII; removed "competition assay"

whereby the proportion of said immobilised ligand bound by TCII or holo-TCII, by said non-immobilised ligand or by complexes of said non-immobilised ligand and TCII or holo-TCII is dependent on the amount of holo-TCII present in said sample, and,

said non-immobilised ligand is capable of generating a directly or indirectly detectable signal when bound or when unbound;

separating a bound fraction from a non-bound fraction; and

directly or indirectly determining a bound fraction consisting of the non-immobilised ligand bound to the immobilised ligand or a non-bound fraction consisting of the non-immobilised ligand non-bound and in solution;

where the contacting of the sample and said non-immobilised ligand with the solid support may be performed simultaneously, separately in either order, or sequentially in either order. 35

58(new). An assay method as claimed in claim 50 wherein said specific binding ligand binds holo-TCII with an affinity constant of at least $10^9 M^{-1}$. p 36

59(new). An assay method as claimed in claim 50 wherein said specific binding ligand binds holo-TCII with an affinity constant of greater than $10^{11} M^{-1}$. 37

60(new). An assay method as claimed in claim 50 wherein the degree of cross-reactivity of said specific binding ligand with HC is between 0.1% and 1%. 38

61(new). An assay method as claimed in claim 50 wherein the degree of cross-reactivity of said specific binding ligand with HC is less than 0.1%. 39

62(new). An assay method as claimed in claim 50 wherein said sample which has been contacted with the immobilized cobalamin or analogue or fragment thereof is 40

further contacted with a solid phase support having immobilized thereon said specific binding ligand and to which is bound a labelled ligand recognizing the same binding sites on the immobilized specific binding ligand as holo-TCII, whereby holo-TCII in said sample competes with said bound labelled ligand for said binding sites such that after equilibration of the system there is a directly proportional relationship between the amount of labelled ligand displaced from said solid phase support and detectable in solution and the amount of holo-TCII present in the original sample; said labelled ligand being detected directly or indirectly as the amount of labelled ligand bound or not bound to said solid phase support as appropriate.

63(new). An assay method as claimed in claim 50 wherein said sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof is further contacted with a solid phase support having holo-TCII immobilised thereon and with a labelled non-immobilised holo-TCII specific binding ligand, whereby free holo-TCII in the sample and immobilised holo-TCII compete for binding to the labelled non-immobilised ligand; and determination of the labelled ligand bound to the solid phase support or remaining in solution allows determination of the holo-TCII concentration. 41

64(new). An assay method as claimed in claim 50 wherein said sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof is further contacted with labelled holo-TCII and an immobilised ligand therefor whereby labelled and non-labelled holo-TCII compete for binding to the immobilised ligand and after equilibrium is reached, the amount of labelled holo-TCII bound to the immobilised ligand is indirectly proportional to the amount of holo-TCII in the sample. 42

65(new). An assay method as claimed in claim 50 wherein said body sample is a sample selected from the group consisting of seminal fluid, cerebro-spinal fluid, amnioticfluid and a blood derived sample. 43

66(new). An assay as claimed in claim 65 wherein said blood derived sample is serum or plasma. 44

67(new). An assay method as claimed in claim 50 wherein said bound fraction is separated from said unbound fraction by precipitation, centrifugation, filtration or chromatographic methods. 45

68(new). An assay method as claimed in claim 50 wherein said ligand is labelled with a signal forming label which may be determined by luminescence, chemiluminescence, colorimetric assessment, fluorescence, radioactivity or by enzymic activity. 46

69(new). An assay method as claimed in claim 50 in which assay calibration is effected using a holo-TCII standard. 47

70(new). An assay as claimed in claim 69 wherein said standard is human, native or recombinant holo-TCII. 48

71(new). An assay method for the determination of holo-transcobalamin II (holo-TCII) in a body sample, comprising: contacting a cell free sample of a body fluid with an immobilized cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of transcobalamin II (TCII) and haptocorrin over the holo-forms thereof; removing the bound apo-forms from the sample; subsequently contacting said sample with an immobilised or immobilizable specific binding ligand for TCII or holo-TCII; separating a ligand bound fraction from a non-ligand bound fraction; and measuring the holo-TCII content of said ligand bound fraction. 49

72(new). An assay method as claimed in claim 50 wherein said method comprises contacting a solid support having immobilised thereon said specific binding

ligand for TCII or holo-TCII, with a non-immobilised ligand and also with the sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof,

wherein said immobilised ligand binds to TCII or holo-TCII, to said non-immobilised ligand or to complexes of said TCII or holo-TCII and said non-immobilised ligand, and said non-immobilised ligand binds to at least one of said immobilised ligand, TCII or holo-TCII and complexes of said immobilised ligand apo-TCII or holo-TCII;

wherein said assay is a competition assay and said immobilised ligand is specific for holo-TCII and competitors thereof;

whereby the proportion of said immobilised ligand bound by TCII or holo-TCII, by said non-immobilised ligand or by complexes of said non-immobilised ligand and TCII or holo-TCII is dependent on the amount of holo-TCII present in said sample, and,

said non-immobilised ligand is capable of generating a directly or indirectly detectable signal when bound or when unbound;

separating a bound fraction from a non-bound fraction; and

directly or indirectly determining a bound fraction consisting of the non-immobilised ligand bound to the immobilised ligand or a non-bound fraction consisting of the non-immobilised ligand non-bound and in solution;

where the contacting of the sample and said non-immobilised ligand with the solid support may be performed simultaneously, separately in either order, or sequentially in either order.

09/679,043

method I
claim 50

contacting cell-free sample w/ immobilised cobalamin to bind apo forms of TCII and HC.

↓

add non-immobilized specific binding ligand for TCII or holo TCII

Separating (ligand-bound) TC II or holo TC II

Separating (ligand non-bound) TC II or holo TCII * unbound

measureing TC II or cobalamin to determine apo TC II.

435 7.1

removal of apo TC-II or apo-TC II unable to bind SPL for TCII or holo TC II.

method II
claim 71.

immobilized specific binding ligand for TC II or holo-TC II

435. 7.92

method III
Claims 50 + 72 immobilized specific binding ligand + sample immobilized w/ cobalamin competitors. analysis.

non-immobilised ligand

↓ combination of competitors in solution.

435 7.93

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claims 1-27 (canceled).

28(New). An assay method for the determination of transcobalamin II bound cobalamin (holo-TCII) in a body sample, comprising contacting a cell free sample of a body fluid with an immobilized cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of TC II and haptocorrin in said sample, subsequently contacting said sample which has been contacted with the immobilized cobalamin or analogue or fragment thereof, with a specific binding ligand for TC II or holo-TC II, separating a ligand bound fraction from a non-ligand bound fraction and measuring the TC II or cobalamin content of said ligand bound fraction to determine the quantity of holo-TCII in the body sample being assayed.

29(New). An assay method as claimed in claim 28 wherein the separation of said ligand bound fraction from said non-ligand bound fraction is so performed that the holo-TCII concentration is increased by at least 3-fold.

30(New). An assay method as claimed in claim 28 wherein said assay is capable of detecting holo-TCII at a concentration as low as 9 pM.

31(New). An assay method as claimed in claim 28 wherein said specific binding ligand is a polyclonal or monoclonal antibody, an antibody fragment, a polypeptide, an oligopeptide, a small organic chemical, a specific binder selected from a combinatorial chemistry or phage display library, a specifically binding sequence of DNA or RNA, or a cell surface receptor.

32(New). An assay method as claimed in claim 28 wherein said specific binding ligand exhibits a high degree of selectivity and specificity towards TCII and exhibits low affinity towards other transcobalamin proteins, in either apo or holo form, or any other cobalamin-binding protein.

33(New). An assay method as claimed in claim 28 wherein cobalamin in said ligand bound fraction is released from the holo TCII molecules therein by changing the temperature or the pH of the surrounding medium.

34(New). An assay method as claimed in claim 33 wherein said released cobalamin is determined by a competition assay performed by contacting an immobilised binding partner for cobalamin with the released cobalamin of the sample in the presence of labelled ligand which competes with the released cobalamin for binding to the immobilised binding partner. LAB

35(New). An assay method as claimed in claim 28 wherein said method comprises contacting a solid support having immobilised thereon said specific binding ligand for TCII or holo-TCII, with a non-immobilised ligand and also with the sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof,

wherein said immobilised ligand is capable of binding to TCII or holo-TCII, to said non-immobilised ligand or to complexes of said TCII or holo-TCII and said non-immobilised ligand, and said non-immobilised ligand is capable of binding to at least one of said immobilised ligand, TCII or holo-TCII and complexes of said immobilised ligand and TCII or holo-TCII;

wherein if said assay method is a sandwich assay, at least one of said ligands is specific for holo-TCII and if said assay is a competition assay said immobilised ligand is specific for holo-TCII and competitors thereof;

whereby the proportion of said immobilised ligand bound by TCII or holo-TCII, by said non-immobilised ligand or by complexes of said non-immobilised ligand and TCII or holo-TCII is dependent on the amount of holo-TCII present in said sample, and,

said non-immobilised ligand is capable of generating a directly or indirectly detectable signal when bound or when unbound;

separating a bound fraction from a non-bound fraction; and

directly or indirectly determining the non-immobilised ligand bound to the immobilised ligand (the bound fraction) or non-bound and in solution (the non-bound fraction);

where the contacting of the sample and said non-immobilised ligand with the solid support may be performed separately, simultaneously or sequentially, and if performed separately or sequentially, they may be contacted in either order.

36(New). An assay method as claimed in claim 28 wherein said specific binding ligand possesses an affinity constant for binding to holo-TCII of at least $10^9 M^{-1}$.

37(New). An assay method as claimed in claim 28 wherein said specific binding ligand possesses an affinity constant for binding to holo-TCII of greater than $10^{11} M^{-1}$.

38(New). An assay method as claimed in claim 28 wherein the degree of cross-reactivity of said specific binding ligand with HC is between 0.1% and 1%.

39(New). An assay method as claimed in claim 28 wherein the degree of cross-reactivity of said specific binding ligand with HC is less than 0.1%.

✳ 40(New). An assay method as claimed in claim 28 wherein said sample which has been contacted with the immobilized cobalamin or analogue or fragment thereof is further contacted with a solid phase support having immobilized thereon said specific binding ligand and to which is bound a labelled ligand recognizing the same binding

sites on the immobilized specific binding ligand as holo-TCII, whereby holo-TCII in said sample competes with said bound labelled ligand for said binding sites such that after equilibration of the system there is a directly proportional relationship between the amount of labelled ligand displaced from said solid phase support and detectable in solution and the amount of holo-TCII present in the original sample; said labelled ligand being detected directly or indirectly as the amount of labelled ligand bound or not bound to said solid phase support as appropriate.

41(New). An assay method as claimed in claim 28 wherein said sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof is further contacted with a solid phase support having holo-TCII immobilised thereon and with a labelled non-immobilised holo-TCII specific binding ligand, whereby free holo-TC II in the sample and immobilised holo-TCII compete for binding to the labelled non-immobilised ligand; and determination of the labelled ligand bound to the solid phase support or remaining in solution allows determination of the holo-TC II concentration.

42(New). An assay method as claimed in claim 28 wherein said sample which has been contacted with the immobilised cobalamin or analogue or fragment thereof is further contacted with labelled holo-TCII and an immobilised ligand therefor whereby labelled and non-labelled holo-TCII compete for binding to the immobilised ligand and after equilibrium is reached, the amount of labelled holo-TCII bound to the immobilised ligand is indirectly proportional to the amount of holo-TCII in the sample.

43(New). An assay method as claimed in claim 28 wherein said body sample is selected from the group ~~comprising~~ consisting of seminal fluid, cerebro-spinal fluid, amniotic fluid and a blood derived sample.

44(New). An assay as claimed in claim 43 wherein said blood derived sample is serum or plasma.

45(New). An assay method as claimed in claim 28 wherein said bound fraction is separated from said unbound fraction by precipitation, centrifugation, filtration or chromatographic methods.

46(New). An assay method as claimed in claim 28 wherein said ligand is labelled with a signal forming label which may be determined by luminescence, chemiluminescence, colorimetric assessment, fluorescence, radioactivity or by enzymic activity.

47(New). An assay method as claimed in claim 28 in which assay calibration is effected using a holo-TCII standard.

48(New). An assay as claimed in claim 47 wherein said standard is human, native or recombinant holo-TCII.

49(New). An assay method for the determination of holo-transcobalamin II (holo-TCII) in a body sample, comprising: contacting a cell free sample of a body fluid with an immobilized cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of transcobalamin II (TCII) and haptocorrin; removing the bound apo-forms from the sample; subsequently contacting said sample with an immobilised or immobilizable specific binding ligand for TCII or holo-TCII; separating a ligand bound fraction from a non-ligand bound fraction; and measuring the holo-TCII content of said ligand bound fraction.